JOURNAL OF BACTERIOLOGY, Feb. 1994, p. 748–755
0021-9193/94/$04.00+0

Vol. 176, No. 3

FimH Family of Type 1 Fimbrial Adhesins: Functional Heterogeneity due to Minor Sequence Variations among *fimH* Genes

EVGENI V. SOKURENKO,[1] HARRY S. COURTNEY,[2,3] DENNIS E. OHMAN,[3,4] PER KLEMM,[5] AND DAVID L. HASTY[1,3]*

Departments of Anatomy and Neurobiology,[1] Microbiology and Immunology,[4] and Medicine,[2] University of Tennessee, Memphis, Tennessee 38163; Memphis VA Medical Center, Memphis, Tennessee 38104[3]; and Department of Microbiology, Technical University of Denmark, DK-2800 Lyngby, Denmark[5]

Received 23 August 1993/Accepted 19 November 1993

We recently reported that the type 1-fimbriated *Escherichia coli* strains CSH-50 and HB101(pPKL4), both K-12 derivatives, have different patterns of adhesion to yeast mannan, human plasma fibronectin, and fibronectin derivatives, suggesting functional heterogeneity of type 1 fimbriae. In this report, we provide evidence that this functional heterogeneity is due to variations in the *fimH* genes. We also investigated functional heterogeneity among clinical isolates and whether variation in *fimH* genes accounts for differences in receptor specificity. Twelve isolates obtained from human urine were tested for their ability to adhere to mannan, fibronectin, periodate-treated fibronectin, and a synthetic peptide copying the 30 amino-terminal residues of fibronectin. CSH-50 and HB101(pPKL4) were tested for comparison. Selected isolates were also tested for adhesion to purified fragments spanning the entire fibronectin molecule. Three distinct functional classes, designated M, MF, and MFP, were observed. The *fimH* genes were amplified by PCR from chromosomal DNA obtained from representative strains and expressed in a Δ*fim* strain (AAEC191A) transformed with a recombinant plasmid containing the entire *fim* gene cluster but with a translational stop-linker inserted into the *fimH* gene (pPKL114). Cloned *fimH* genes conferred on AAEC191A(pPKL114) receptor specificities mimicking those of the parent strains from which the *fimH* genes were obtained, demonstrating that the FimH subunits are responsible for the functional heterogeneity. Representative *fimH* genes were sequenced, and the deduced amino acid sequences were compared with the previously published FimH sequence. Allelic variants exhibiting >98% homology and encoding proteins differing by as little as a single amino acid substitution confer distinct adhesive phenotypes. This unexpected adhesive diversity within the FimH family broadens the scope of potential receptors for enterobacterial adhesion and may lead to a fundamental change in our understanding of the role(s) that type 1 fimbriae may play in enterobacterial ecology or pathogenesis.

Adhesion of bacteria to host surfaces is commonly regarded as an essential step enabling bacteria to become established as members of the host's normal flora or to cause an infection (7, 18). Bacterial lectins are the most common and most thoroughly studied type of adhesin among both gram-negative and gram-positive bacteria (42). Evolutionary pressures have selected lectins for adhesive functions, probably because of the abundance of glycoconjugates on animate and inanimate surfaces. One class of structures that *Escherichia coli* and other members of the family *Enterobacteriaceae* have evolved to adhere to host glycoproteins in a saccharide-dependent manner are surface fibrils called fimbriae (14), or pili (10).

The most common of the enterobacterial fimbriae, type 1, or mannose-specific (MS), fimbriae (11, 13, 14, 23), are heteropolymers of four different subunits (28, 44). Approximately 1,000 copies of the 17-kDa primary structural subunit, FimA (or PilA), are polymerized into a right-handed helical fibril also containing minor amounts of the FimF, FimG, and FimH subunits (20, 24, 27, 32). The 28-kDa FimH subunit has been shown by direct and indirect tests to be the fimbrial lectin (1–3, 20, 21, 27, 29, 32, 36, 55). The FimA subunit is variable, but FimH is highly conserved antigenically and genetically among enterobacteria (4). Interactions between type 1 fimbriae and D-mannose-containing receptors have been shown in a number of studies to play a key role in the infectious process, but this topic remains somewhat controversial (1, 3, 8, 19, 25, 26, 31, 33, 44, 50).

Detailed analysis of adhesion or agglutination inhibition by various mannosides and manno-oligosaccharides has suggested that the combining site of the type 1 adhesin is in the form of an extended pocket fitting best the trisaccharide α-D-Man*p*-(1–3)-β-D-Man*p*-(1–4)-D-GlcNAc and with a hydrophobic region within or close to the combining site (16). A similar pattern of specificity was found in independent inhibition studies (37) as well as in direct adhesion studies using "neoglycolipids" as receptors (47). The combining site of the *Klebsiella pneumoniae* type 1 adhesin is similar, while that of the *Salmonella typhimurium* type 1 adhesin appears to be smaller and devoid of a hydrophobic region (16). Thus, it has long been thought that type 1 fimbriae of enterobacteria were functionally quite similar and that the primary essential characteristic of any potential receptor was the presence of terminal α1-3-linked mannosyl residues.

Recently we reported that the type 1-fimbriated, K-12-derived *E. coli* CSH-50 exhibits mannose-sensitive peptide-binding activity (51). *E. coli* CSH-50 bound to yeast mannan (Mn), a highly mannosylated glycoprotein, and to human plasma fibronectin (Fn) when immobilized on assay wells.

* Corresponding author. Mailing address: Research Service (151), VA Medical Center, 1030 Jefferson Avenue, Memphis, TN 38104. Phone: (901) 523-8990, ext. 7556. Fax: (901) 577-7273.

Adhesion to Mn but not to Fn was essentially eliminated by periodate treatment. Furthermore, *E. coli* CSH-50 adhered in an MS fashion to nonglycosylated peptide fragments of Fn and to a synthetic peptide copying the first 30 residues of the Fn molecule, FnSp1. A well-characterized recombinant strain expressing type 1 fimbriae of *E. coli* PC31, HB101(pPKL4), adhered to Mn but did not adhere to Fn or to FnSp1, suggesting that *E. coli* type 1 fimbriae are functionally heterogeneous.

Several clinical isolates (CIs) of *E. coli* obtained from human urine also expressed peptide-binding activity similar to CSH-50, indicating that this new phenotype was not restricted to a laboratory strain. Other CIs expressed an adhesive activity similar to that of HB101(pPKL4). A third class of type 1 fimbria-mediated adhesive phenotype was also observed among the CIs. Because the FimH fimbrial subunit is known to be the mannose-specific lectin, we focused our first efforts to understand the heterogeneous receptor specificity on the *fimH* gene. In this report, we describe three functional classes of *E. coli* type 1 fimbriae and show that highly conserved allelic variants of the *fimH* gene are responsible for the functional heterogeneity.

MATERIALS AND METHODS

Reagents. Yeast Mn, a polymannosylated glycoprotein isolated from *Saccharomyces cerevisiae* cell walls, was obtained from a commercial source (Sigma Chemical Co., St. Louis, Mo.). Mn is composed of an N-linked backbone of β1,2-linked mannopyranose units with α-linked mannopyranose side chains (38). The majority of the carbohydrate of human plasma Fn is composed of N-glycosidic complex-type biantennary glycans, and no high-mannose-type or hybrid-type N-glycans have been described (30, 45, 54). Human plasma Fn, Fn fragments, and FnSp1 were purified or synthesized as described previously (5, 15, 51, 58). Periodate treatment was performed as described previously (51). The saccharide content of the four substrates was characterized by using concanavalin A, which reacts with terminal and internal mannosyl residues, and the *Galanthus nivalis* agglutinin, which recognizes only terminal Manα1-3Man, Manα1-6Man, and Manα1-2Man sequences (E. Y. Laboratories, San Mateo, Calif.). Immobilized Mn and Fn both reacted with concanavalin A, whereas *G. nivalis* agglutinin bound only to Mn (data not shown). These results are consistent with the known structures of the oligosaccharide moieties of these two compounds. Neither lectin reacted with immobilized FnSp1. Periodate treatment (51) of Mn or Fn eliminated lectin reactivity (data not shown).

Bacterial strains and plasmids. Strain CSH-50 is a Cold Spring Harbor Laboratory *E. coli* K-12-derived strain (35) that has been used in several previous studies in this laboratory. *E. coli* AAEC191A, a derivative of *E. coli* K-12 strain MG1655, which has had the entire *fim* gene cluster deleted by allelic exchange (9), was generously provided by Ian Blomfield (Bowman-Gray University, Winston-Salem, N.C.). The 12 CIs were urine isolates obtained from the clinical microbiology laboratories of the Memphis VA Medical Center or the City of Memphis Hospitals, Memphis, Tenn., selected on the basis of MS agglutination of yeast cells after growth in broth, a classic test for type 1 fimbriae. Plasmid pPKL4, a pBR322 derivative containing the entire *fim* gene cluster from *E. coli* K-12 strain PC31, has been described previously (28). pPKL114 is a recombinant plasmid derived from pPKL4 but with a translational stop-linker inserted into the *Kpn*I site in the *fimH* gene. No transcriptional effects of the stop-linker are to be expected.

PCR. Oligonucleotide primers were designed from the published sequence for the *fimH* gene in pPKL4 (27) (GenBank accession number X05672). The 5′ primers copied regions 13 and 49 bp upstream from the *fimH* gene and were extended on the 5′ end by an *Apa*LI restriction site and a GC clamp: primer 1, 5′-GGGGG GTGCAC ACC TAC AGC TGA ACC CGG-3′; primer 2, 5′-GGGG GTGCAC T CAG GGA ACC ATT CAG GCA-3′. The 3′ primers copied 18 bases of the bottom strand of the *fimH* gene that encode the six terminal amino acids of FimH and were extended by an *Fsp*I or *Sph*I site and a GC clamp: primer 3, 5′-GGG TGCGCA TTA TTG ATA AAC AAA AGT CAC-3′; primer 4, 5′-GGG GCATGC TTA TTG ATA AAC AAA AGT CAC-3′. PCR was performed in a Perkin-Elmer Cetus automatic thermal cycler with denaturation at 96°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 2 min, for a total of 40 cycles. Purification, restriction, and ligation of DNA were done by standard procedures (39, 48). All primers for PCR and for nucleotide sequencing were produced by the Molecular Resources Center, University of Tennessee, Memphis.

Subcloning. PCR products were inserted into the *Apa*LI-*Sph*I region of plasmid pGB2-24, constructed as shown in Fig. 1. We initially chose pACYC177 as a cloning vector for *fimH* but had to subclone *fimH* from pACYC177 because of a high frequency of appearance of spontaneous Km^r in the AAEC191A host strain. The origin of this problem is not entirely clear. Plasmid pGB2-24 and its derivatives were compatible with the pBR322-based plasmid pPKL114 used for complementation experiments. All recombinant strains tested thus far by this technique are stable, and all exhibit the same adhesive phenotype as the parent strains from which the *fimH* genes were cloned.

Construction of chimeric *fimH* genes. Unique restriction sites (Fig. 2) were used to construct chimeric *fimH* genes with the pPKL4 *fimH* gene as the genetic background and exchange of restriction fragments obtained from the newly described *fimH* genes. Various fragments covering the entirety of each new *fimH* gene were ligated into appropriate "spaces" created in the pPKL4 *fimH* gene in pGB2-24. Each chimera was analyzed by restriction mapping, and the nucleotide sequences of bridging segments were determined to ensure proper constructions. Plasmids containing chimeric *fimH* genes were transformed into AAEC191A(pPKL114) and tested in adhesion assays.

Nucleotide sequencing. The nucleotide sequences of *fimH* genes were determined by the dideoxynucleotide chain termination method of Sanger et al. (49) with Sequenase II (U.S. Biochemical Corp., Cleveland, Ohio) or the *fmol* Polymerase Sequencing System (Promega, Madison, Wis.) and [α-^{35}S] dATP (800 to 1,000 Ci/mmol) according to the manufacturers' suggestions. The amino acid sequences were deduced from nucleotide sequences by using MacVector protein and DNA analysis software (Eastman Chemical Co., New Haven, Conn.). To ensure fidelity of the PCR amplification, selected *fimH* genes were reamplified, cloned, tested for activity, and resequenced.

Yeast cell aggregation assay. *E. coli* strains were tested for their ability to aggregate yeast cells. Commercial baker's yeast (*Saccharomyces cerevisiae*) was suspended in phosphate-buffered saline (PBS; 5 mg [dry weight]/ml). *E. coli* strains were washed in PBS, resuspended to an optical density at 530 nm (OD_{530}) of 0.4, and mixed with the yeast cell suspension in PBS with or without 1% D-mannose. Aggregation was monitored visually, and the titer was recorded as the last dilution giving a positive aggregation reaction.

Adhesion assays. Adhesion assays were performed as de-

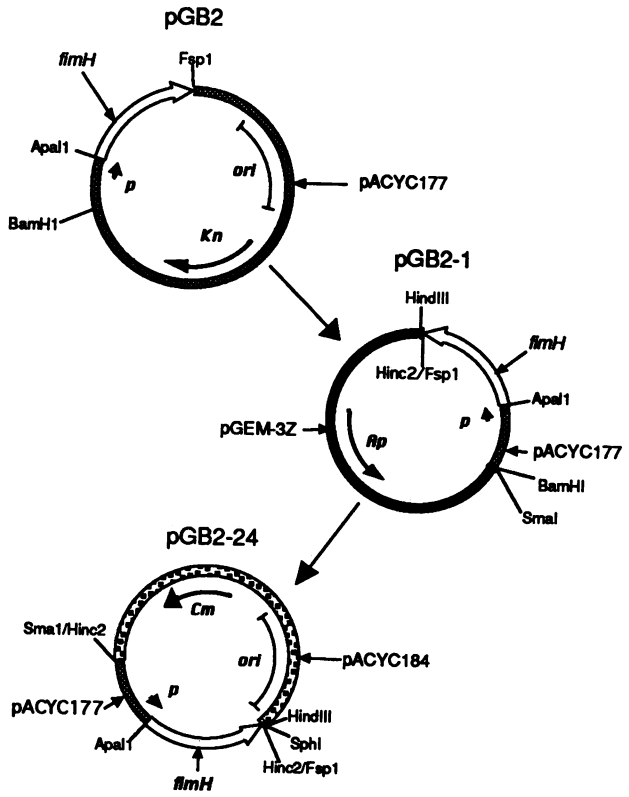

FIG. 1. Schematic model for the construction of recombinant plasmid pGB2-24 used for transforming *E. coli* AAEC191A(pPKL114) with cloned *fimH* genes. The PCR product from pPKL4 was cut with restriction enzymes and ligated into the *Apa*LI and *Fsp*I restriction sites of plasmid pACYC177 (New England Biolabs, Beverly, Mass.), creating plasmid pGB2. The insert and upstream region of pACYC177 containing the *bla* promoter was cut from pGB2 with *Fsp*I and *Bam*HI and subcloned into the polylinker site of pGEM-3Z (Promega, Madison, Wis.) that had been cut with *Bam*HI and *Hinc*II, creating plasmid pGB2-1. The insert was cut out again with *Sma*I and *Hind*III and subcloned into pACYC184 (New England Biolabs) cut with *Hinc*II and *Hind*III, creating plasmid pGB2-24, containing the *fimH* gene from pPKL4. Cutting the *fimH* gene from pGB2-24 with *Apa*LI and *Sph*I makes it possible to easily insert other amplified *fimH* genes.

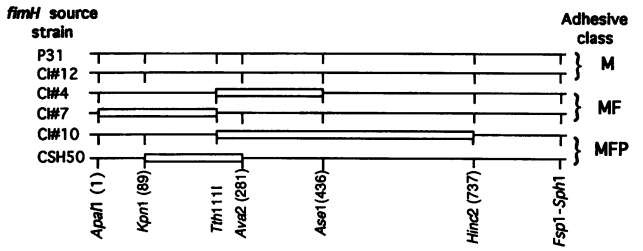

FIG. 2. Restriction map of *fimH* genes. Five unique restriction sites are present in the PC31 *fimH* gene. Numbers in parentheses following enzymes are the base pair positions of the cut sites. Some of these sites are found in the other *fimH* genes, as marked. Chimeric genes were produced by exchanging each available restriction fragment from the other five *fimH* genes with corresponding fragments in the PC31 gene, and recombinant strains expressing the resulting chimeric FimH subunits were tested for adhesion. Fragments indicated by boxes are those which conferred MF or MFP adhesive phenotypes on the chimeric genes (see text).

scribed previously (51). Briefly, microtiter assay wells were coated with receptor molecules, blocked with bovine serum albumin (BSA)-PBS, washed two times with PBS, and incubated with bacterial suspensions in 0.1% BSA–PBS. After incubation, wells were washed and adherent bacteria were detected by using rabbit anti-*E. coli* serum followed by peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG). Reaction product generated from the 5-aminosalicylic acid substrate was measured after 10 to 15 min in an automatic microplate reader (Molecular Devices, Inc., Menlo Park, Calif.). The values reported are corrected for background reaction with BSA-coated plates as controls.

RESULTS

We reported previously that the type 1 fimbriae of *E. coli* CSH-50 and HB101(pPKL4) differ functionally in their pattern of adhesion to Mn, Fn, periodate-treated Fn, and a synthetic peptide, FnSp1, immobilized on plastic (51). Since CSH-50 and HB101(pPKL4) are laboratory strains, we tested 12 CIs of *E. coli* from human urine for adhesion to these substrates. All of the CIs agglutinated yeast cells in an MS fashion. Five of the 12 CIs adhered only to Mn. HB101(pPKL4) and CI 12 are shown as examples of this class, tentatively designated the M class, in Fig. 3A. Three of the 12 CIs adhered to Mn and Fn but not to periodate-treated Fn or to FnSp1. CIs 4 and 7 are shown as examples of this class, designated the MF class, in Fig. 3A. Three of the 12 CIs adhered to each of the substrates. CSH-50 and CI 10 are shown as examples of this class, designated the MFP class in Fig. 3A. FnSp1 inhibits adhesion of MFP class strains to Mn, but M and MF class adhesion to Mn is unaffected by the synthetic peptide.

Adhesion of strains representing these three classes to Fn fragments further illustrated the distinct differences between the three classes. The M class CI 12 does not adhere to any of the Fn fragments (Fig. 4). The MF class CI 4 adheres to the 40-kDa gelatin-binding fragment. The MFP class CI 10 adheres, with only slight differences, to all five fragments of Fn tested. Periodate treatment eliminated binding of CI 4 to domain 2 but had no effect on the binding of CI 10 to any of the Fn domains (data not shown).

Since the FimH subunit has been shown to mediate the mannose-sensitive activity of type 1 fimbriae, we focused our initial efforts on elucidating the molecular basis for the observed functional heterogeneity on the *fimH* gene. *fimH* genes were amplified from chromosomal (or plasmid, for pPKL4) DNA, and the genes were cloned into pACYC177 and subcloned into pACYC184 under control of the β-lactamase promoter of pACYC177, as detailed in Materials and Methods (Fig. 1).

The adhesive phenotypes conferred by the *fimH* genes were tested in the following way. *E. coli* K-12 strain AAEC191A (Δ*fim*) was first transformed with plasmid pPKL114, which contains an intact *fim* gene cluster but with a translational stop-linker inserted into the last gene, *fimH*. This derivative produces morphologically normal fimbriae that are nonadhesive because of the absence of the FimH subunit. Plasmids harboring cloned *fimH* genes were transformed into *E. coli* AAEC191A(pPKL114), and the resultant strains were tested for their ability to adhere to Mn, Fn, periodate-treated Fn, and FnSp1 (Fig. 3B). Each of the recombinant strains displayed adhesive phenotypes mimicking those of the parent strains from which the *fimH* genes were obtained. *fimH* genes were cloned from each of the other eight CIs (data not shown), and similar results were obtained, with the adhesion of recombinant strains mimicking that exhibited by the parental CIs.

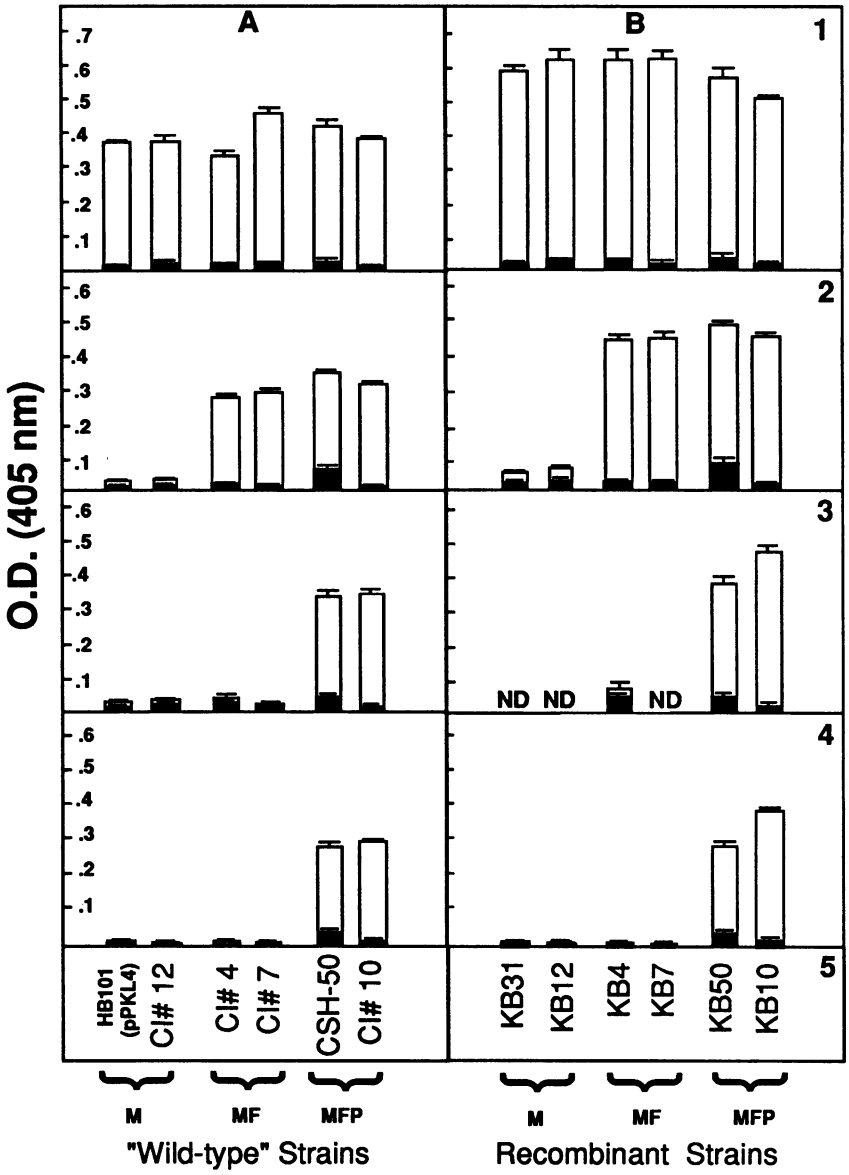

FIG. 3. Adhesion of representative "wild-type" (A) and recombinant (B) M, MF, and MFP class strains to Mn (panels 1), Fn (panels 2), periodate-treated Fn (panels 3), and FnSp1 (panels 4). Strain designations for the wild-type strains are given in panel A5. The strain designations given in panel B5 (KB31, KB12, KB4, KB7, KB50, and KB10) are for recombinant strains of AAEC191A(pPKL114), which is a *fimH* mutant, after transformation with plasmids that contain *fimH*$^+$ from strain HB101(pPKL4), CI 12, CI 4, CI 7, CSH-50, and CI 10, respectively. Open columns, bacteria incubated without D-mannose; solid columns, bacteria incubated with D-mannose. Values are the means ± standard error of the mean (n = 4) for each column. ND, not determined.

The complete nucleotide sequences of each of the six representative *fimH* genes were determined, and the amino acid sequences of the FimH proteins were deduced (Fig. 5). The nucleotide and deduced amino acid sequences of the pPKL4 *fimH* gene reported previously (27) showed an arginine at position 176, but the present analysis shows that this residue is a proline. Otherwise, the sequences are identical. Sequencing was repeated on several independently amplified and cloned *fimH* genes, those from pPKL4, CI 10, and CI 7, to confirm sequence fidelity and ensure that there were no PCR-induced errors, and none were found.

The nucleotide and deduced amino acid sequences of the *fimH* alleles are >98% conserved, but there is more than one amino acid residue difference in all but one of the new FimH sequences compared with the published pPKL4 sequence. To focus on the sequence differences that resulted in changes in functional activity, we took advantage of unique restriction sites (Fig. 2) to construct chimeric *fimH* genes. Multiple restriction fragments covering the entirety of each of the sequenced *fimH* genes were exchanged with corresponding fragments in the prototypical *fimH* gene of *E. coli* PC31, which was amplified from pPKL4, cloned into pACYC184, and used as the genetic background. Recombinant plasmids containing the chimeric *fimH* genes were transformed into *E. coli* AAEC191A(pPKL114), and transformants were tested for adhesive phenotype, allowing determination of the regions of

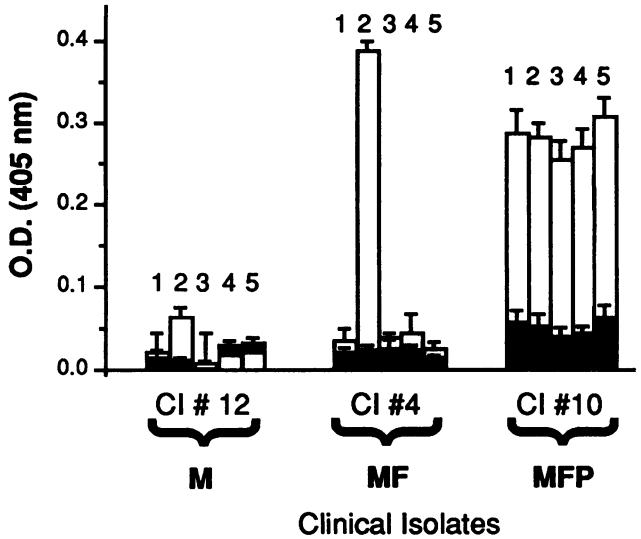

FIG. 4. Adhesion of representative M, MF, and MFP class strains (CIs 12, 4, and 10, respectively) to Fn fragments. Columns 1 to 5 indicate adhesion to the NH_2-terminal 30-kDa domain, the 55-kDa gelatin-binding domain, the 110-kDa cell attachment domain, the 29- to 38-kDa heparin-binding domains, and the 20-kDa COOH-terminal domain, respectively (see reference 51). Open columns, adhesion in the absence of D-mannose; solid columns, adhesion in the presence of D-mannose. Values are means ± standard error of the mean (n = 4).

each gene capable of conferring functional activity (Fig. 2). All of the sequence changes that affected function occurred between residues 33 and 119 of the 279-residue mature FimH sequence.

DISCUSSION

The FimH subunit is the D-mannose-sensitive adhesin of type 1 fimbriae, adhesive organelles common to the *Enterobacteriaceae*. It is widely accepted that host receptors for type 1 fimbriae are strictly limited to glycoproteins containing terminal mannosyl residues (16, 37, 40, 41, 43, 47), but this generalization is not correct. Allelic variants of *E. coli fimH* genes encoding proteins differing by as little as a single amino acid confer distinct adhesive phenotypes. Surprisingly, active receptors include glycoprotein domains where mannosyl residues are not terminal and even protein domains devoid of saccharide.

The functional heterogeneity that we describe must be due entirely to allelic variants of the *fimH* gene, since this is the only variable in these recombinant strains. All other genes necessary for fimbrial subunit synthesis, transport, and assembly are the same in each case. Since the ratios of the various genes and gene products should also be identical, subunit incorporation into the fimbrial superstructure should not vary significantly. These results emphasize that the FimH subunit is the primary determinant of receptor specificity. Other investigators have speculated, however, that the FimA subunit may also affect receptor specificity (55).

In comparing the new FimH sequences with the one published previously (27), the only structural alteration that can be clearly linked to a functional change is the nonconservative substitution of arginine at position 58 in the MFP class CSH-50 FimH subunit for leucine in the M class PC31 FimH subunit. Since each of the other FimH sequences had more than one change, we had to construct chimeric genes to focus on functionally relevant changes. In the case of the MFP class CI 10 FimH, MFP adhesive activity is conferred by a downstream region of the gene from which 12 bp are deleted. It remains to be determined how two distinctly different structural changes result in similar receptor specificities.

The *Apa*LI-*Tth*111I fragment of the CI 7 *fimH* gene confers MF class activity in the CI 7/PC31 *fimH* chimera. Since the asparagine 16 to threonine substitution in the leader sequence is not represented in the mature protein, the histidine 33 to asparagine substitution must be of functional importance for the MF class CI 7 FimH. Comparison of the active regions of the MF class CI 4 and the M class CI 12 FimH subunits suggests the importance of the glutamic acid 73 to glycine substitution for MF class activity of the CI 4 FimH. Thus, histidine 33, arginine 58, glutamic acid 73, and deleted glycine 116 through isoleucine 119 appear to be key residues in the functional activity of the FimH subunits of CI 7, CSH-50, CI 4, and CI 10, respectively. A more precise demonstration of which residues are involved and how they affect the ligand-binding cleft(s) remains to be performed.

At first glance, the MS protein-binding activity of type 1 fimbriae is the most surprising of the adhesive phenotypes described here. The protein-binding activity of FimH (i.e., PilE) subunits was noted earlier in a study characterizing *mutT*-induced mutations in the *fimH* (*pilE*) gene (22), but it was not MS. We do not know whether the protein-binding activity that we have described is in addition to or separate from the mannose-binding activity, but the concept of bifunctional properties of lectins has been established for several years (6). While the MFP class type 1 fimbriae appear to react somewhat promiscuously with most Fn fragments, the reaction does not appear to be nonspecific. For instance, the MFP class CSH-50 type 1 fimbriae do not adhere well to gelatin (51), and a number of synthetic peptides tested did not support adhesion (51a). Furthermore, adhesion to ovalbumin is sensitive to both periodate and glycosidase treatment (51). Further experiments testing a wider range of peptides will be required to determine the consensus amino acid motif that reacts with this class of FimH subunit.

Previous studies suggested that the combining site of the *E. coli* FimH adhesin is in the form of an extended pocket corresponding to the size of a trisaccharide with an associated hydrophobic region (16). All of the adhesive interactions that we have described are MS, but it remains to be determined whether the mannose effect is direct or allosteric. Conformational changes that frequently occur in lectins upon binding the saccharide ligand (46) could affect a second binding site. Site-directed mutations are unlikely to shed much light on how structural changes actually relate to the ligand-binding cleft(s), and it will ultimately be necessary to determine the three-dimensional structure of FimH or FimH fragments crystallized in the presence of ligand to fully understand the structure-function relationships.

The three adhesive classes identified here may understate the functional heterogeneity of type 1 fimbriae. The number of CIs and receptor molecules that we have tested to date is small. A larger group of isolates tested against additional receptor candidates might yield additional functional classes. Preliminary studies with MS *Enterobacter aerogenes* and *K. pneumoniae* strains exhibiting MF class and MFP class activity suggest that heterogeneous receptor specificities will also be found among other type 1-fimbriated enterobacterial species (51a). It is also possible that certain naturally occurring FimH subunits may confer mannose-resistant adhesive activity. The possibility that the MS lectin-like properties of FimH might be eliminated while other adhesive properties of FimH are re-

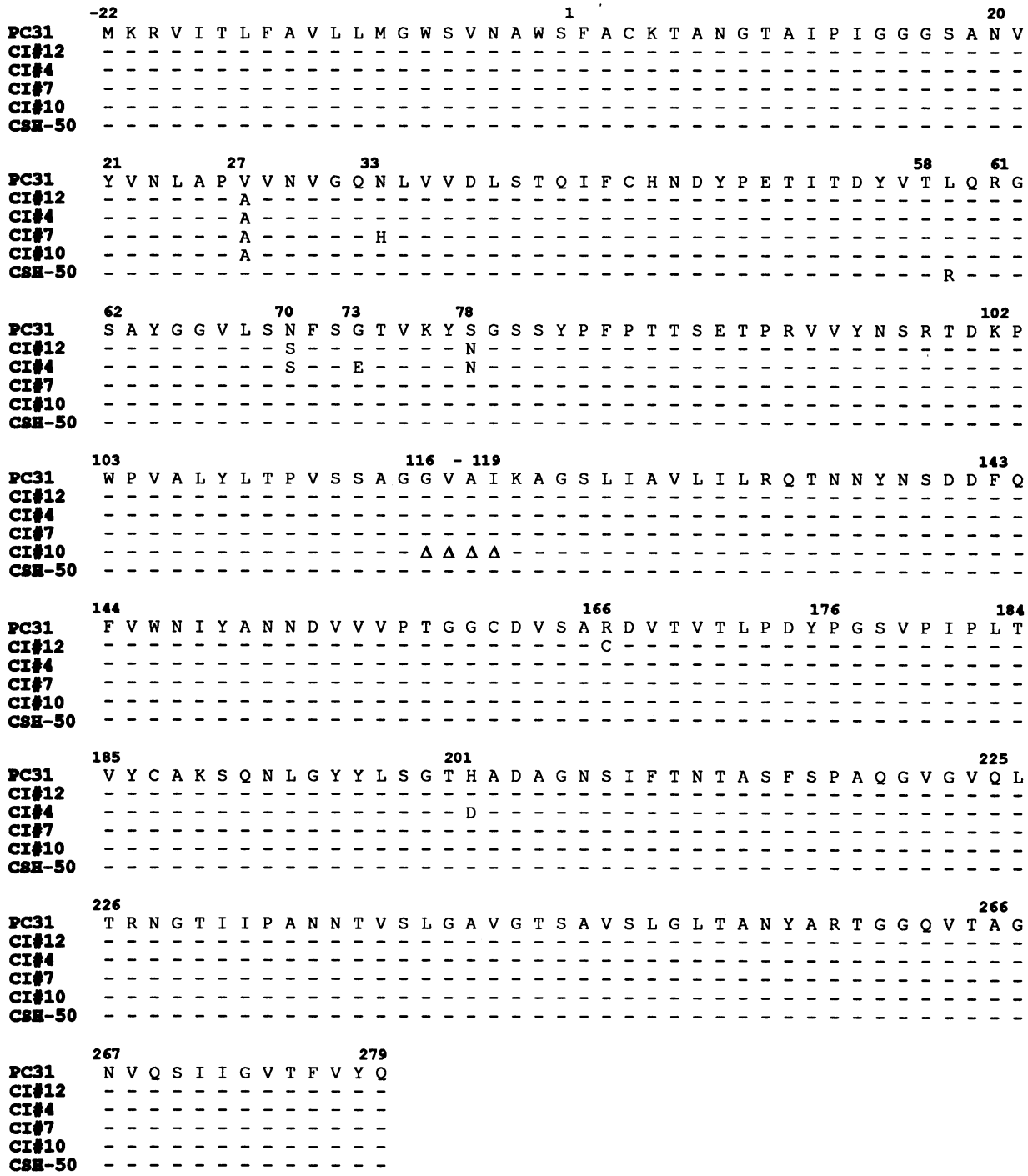

FIG. 5. Amino acid sequences of the FimH subunits deduced from the nucleotide sequences of the *fimH* genes. The standard one-letter code was used. Deleted amino acid residues are indicated by Δ.

tained (e.g., pellicle formation) has been shown previously in a study characterizing *mutT*-induced mutations in the *fimH* (*pilE*) gene (22). Tests for type 1 fimbriation should include additional functional characterization beyond simple sensitivity to mannose. While all type 1 fimbria-mediated adhesion that we have described is mannose sensitive, it is not all mannose or even saccharide specific, as has commonly been thought. Further studies of type 1 fimbriae as a virulence factor must be able to distinguish among the various functional classes.

Allelic variation of the so-called G adhesins of P-fimbriated uropathogenic *E. coli* also results in different functional classes, but the requirement for the Galα1-4Gal sequence within isoreceptors is maintained (52, 53). These differences in G adhesin receptor specificity appear to be rather subtle compared with the differences in FimH receptor specificities,

yet there is significantly greater sequence homology among the *fimH* genes than among the G adhesin genes, some of which have less than 50% homology. The G adhesin receptor specificities affect host susceptibility, largely because of host-specific expression of glycolipid isoreceptor variants. Whether the FimH family of adhesins bears a similar relationship to host susceptibility or tissue tropism remains to be determined. The lectin-independent affinity of P fimbriae for immobilized Fn is not dependent on the G adhesin but on two other minor subunits, E and F, neither of which bears significant homology to FimH (56, 57).

Most *E. coli*, indeed most members of the *Enterobacteriaceae*, are genetically capable of producing type 1 fimbriae, but the pathogenic significance of fimbriae so commonly expressed among enterobacteria occupying distinctly different niches in the host has often been dismissed. Various reports have either supported or questioned the importance of type 1 fimbriae, so the topic remains controversial (1, 8, 19, 25, 26, 31, 33, 34, 42, 50). Nevertheless, the virtual ubiquity of type 1 fimbriae indicates that these fimbriae must serve an essential purpose. Precisely how the newly described family of FimH adhesins may affect our view of the roles that have been suggested for type 1 fimbriae should be investigated. The degree of functional heterogeneity of type 1 fimbriae reported here was unknown when the studies cited above were performed. While a number of basic questions remain to be answered, it should be clear that structural and functional heterogeneity occurs within the class of adhesive organelles commonly referred to as MS or type 1 fimbriae and that the adhesive diversity will lead to a broader spectrum of receptive surfaces for potential colonization. The discovery of the FimH family of adhesins may prove to be an important step toward unravelling the role(s) that type 1 fimbriae may play in the ability of enterobacteria to reach their normal habitat or gain entry into deeper tissues, where devastating effects can occur.

ACKNOWLEDGMENTS

We thank J. Hurley and L. Hatmaker for technical assistance and I. Blomfield, Department of Microbiology and Immunology, Bowman Gray School of Medicine, Winston-Salem, N.C., for kindly providing *E. coli* AAEC191A. We thank Itzhak Ofek and James B. Dale for stimulating discussions and for suggestions regarding the concepts, the experiments, and the manuscript.

This work was supported by NIH grants DE-07218 (D.L.H.) and AI-19146 (D.E.O.), by VA Medical Research funds (D.L.H. and D.E.O.), and by the Danish Technical and Medical Research Councils (P.K.). Dr. Sokurenko is on leave from the Laboratory of Biomedical Technologies, Moscow Sechenov Medical Academy, Moscow, Russia.

REFERENCES

1. **Abraham, S. N., J. P. Babu, C. S. Giampapa, D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1985. Protection against *Escherichia coli*-induced urinary tract infections with hybridoma antibodies directed against type 1 fimbriae or against complementary D-mannose receptors. Infect. Immun. **48:**625–628.
2. **Abraham, S. N., J. D. Goguen, D. Sun, P. Klemm, and E. H. Beachey.** 1987. Identification of two ancillary subunits of *Escherichia coli* type 1 fimbriae by using antibodies against synthetic oligopeptides of *fim* gene products. J. Bacteriol. **169:**5530–5536.
3. **Abraham, S. N., D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1983. Antiadhesive properties of a quaternary structure-specific hybridoma antibody against type 1 fimbriae of *Escherichia coli*. J. Exp. Med. **158:**1114–1128.
4. **Abraham, S. N., D. Sun, J. B. Dale, and E. H. Beachey.** 1988. Conservation of the D-mannose-adhesion protein among type 1 fimbriated members of the family *Enterobacteriaceae*. Nature (London) **336:**682–684.
5. **Akiyama, S. K., and K. M. Yamada.** 1985. The interaction of plasma fibronectin with fibroblastic cells in suspension. J. Biol. Chem. **260:**4492–4500.
6. **Barondes, S. H.** 1988. Bifunctional properties of lectins: lectins redefined. Trends Biochem. Sci. **13:**480–482.
7. **Beachey, E. H.** 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143:**325–345.
8. **Bloch, C. A., B. A. D. Stocker, and P. Orndorff.** 1992. A key role for type 1 pili in enterobacterial communicability. Mol. Microbiol. **6:**697–701.
9. **Blomfield, I. C., M. S. McClain, and B. I. Eisenstein.** 1991. Type 1 fimbria mutants of *Escherichia coli* K-12: characterization of recognized afimbriate strains and construction of new *fim* deletion mutants. Infect. Immun. **5:**1439–1445.
10. **Brinton, C. C., Jr.** 1959. Nonflagellar appendages of bacteria. Nature (London) **183:**782–786.
11. **Brinton, C. C., Jr.** 1965. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram-negative bacteria. Trans. N.Y. Acad. Sci. **27:**1003–1005.
12. **Dodd, D. C., and B. I. Eisenstein.** 1982. Antigenic quantitation of type 1 fimbriae on the surface of *Escherichia coli* cells by an enzyme-linked immunosorbent inhibition assay. Infect. Immun. **38:**764–773.
13. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of *Enterobacteriaceae*, p. 185–217. *In* E. H. Beachey (ed.), Bacterial adherence (Receptors and Recognition Series B, vol. 6). Chapman and Hall, London.
14. **Duguid, J. P., J. W. Smith, G. Dempster, and P. N. Edmunds.** 1955. Non-flagellar filamentous appendages ("fimbriae") and hemagglutinating activity in *Bacterium coli*. J. Pathol. Bacteriol. **70:**335–354.
15. **Engvall, E., and E. Ruoslahti.** 1977. Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. J. Cancer **20:**1–5.
16. **Firon, N., I. Ofek, and N. Sharon.** 1983. Carbohydrate specificity of the surface lectins of *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhimurium*. Carbohydr. Res. **120:**235–249.
17. **Gerlach, G.-F., S. Clegg, and B. L. Allen.** 1989. Identification and characterization of the genes encoding the type 3 and type 1 fimbrial adhesins of *Klebsiella pneumoniae*. J. Bacteriol. **171:**1262–1270.
18. **Gibbons, R. J.** 1984. Microbial ecology. Adherent interactions which may affect microbial ecology in the mouth. J. Dent. Res. **63:**378–385.
19. **Guerina, N. G., T. W. Kessler, V. J. Guerina, M. R. Neutra, H. W. Clegg, S. Langerman, F. A. Scannapieco, and D. A. Goldman.** 1983. The role of pili and capsule in the pathogenesis of neonatal infection with *Escherichia coli* K1. J. Infect. Dis. **148:**395–405.
20. **Hanson, M. S., J. Hempel, and C. C. Brinton, Jr.** 1988. Purification of the *Escherichia coli* type 1 pilin and minor pilus proteins and partial characterization of the adhesin protein. J. Bacteriol. **170:**3350–3358.
21. **Hanson, M. S., and C. C. Brinton, Jr.** 1988. Identification and characterization of the *Escherichia coli* type 1 pilus tip adhesin protein. Nature (London) **322:**265–268.
22. **Harris, S. L., D. A. Elliott, M. C. Blake, L. M. Must, M. Messenger, and P. E. Orndorff.** 1990. Isolation and characterization of mutants with lesions affecting pellicle formation and erythrocyte agglutination by type 1 piliated *Escherichia coli*. J. Bacteriol. **172:**6411–6418.
23. **Hornick, D. B., B. L. Allen, M. A. Horn, and S. Clegg.** 1991. Fimbrial types among respiratory isolates belonging to the family *Enterobacteriaceae*. J. Clin. Microbiol. **29:**1795–1800.
24. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli*. Infect. Immun. **33:**933–938.
25. **Hultgren, S. J., T. N. Porter, A. J. Schaeffer, and J. L. Duncan.** 1985. Role of type 1 pili and effects of phase variation on lower urinary tract infections produced by *Escherichia coli*. Infect. Immun. **50:**370–377.
26. **Keith, B., L. Maurer, P. Spears, and P. Orndorff.** 1986. Receptor-

binding function of type 1 pili effects bladder colonization by a clinical isolate of *Escherichia coli*. Infect. Immun. **53**:693–696.
27. **Klemm, P., and G. Christensen.** 1987. Three *fim* genes required for the regulation of length and mediation of adhesion of *Escherichia coli* type 1 fimbriae. Mol. Gen. Genet. **208**:439–445.
28. **Klemm, P., B. J. Jorgensen, I. van Die, H. de Ree, and H. Bergmans.** 1985. The *fim* genes responsible for synthesis of type 1 fimbriae in *Escherichia coli*: cloning and genetic organization. Mol. Gen. Genet. **199**:410–414.
29. **Krogfelt, K. A., H. Bergmans, and P. Klemm.** 1990. Direct evidence that the FimH protein is the adhesin of *Escherichia coli* type 1 fimbriae. Infect. Immun. **58**:1995–1998.
30. **Krusius, T., M. Fukuda, A. Dell, and E. Ruoslahti.** 1985. Structure of the carbohydrate units of human amniotic fluid fibronectin. J. Biol. Chem. **260**:4110–4116.
31. **Lockman, H. A., and R. Curtiss III.** 1992. Virulence of non-type 1 fimbriated and nonfimbriated, nonflagellated *Salmonella typhimurium* mutants in murine typhoid fever. Infect. Immun. **60**:491–496.
32. **Maurer, L., and P. Orndorff.** 1987. Identification and characterization of genes determining receptor binding and pilus length of *Escherichia coli* type 1 pili. J. Bacteriol. **169**:640–645.
33. **Mayaan, M., I. Ofek, O. Medalia, and M. Aronson.** 1985. Population shift in mannose-specific fimbriated phase of *Klebsiella pneumoniae* during experimental urinary tract infection in mice. Infect. Immun. **49**:785–789.
34. **McCormick, B. A., D. P. Franklin, D. C. Laux, and P. C. Cohen.** 1989. Type 1 pili are not necessary for colonization of the streptomycin-treated mouse large intestine by type 1-piliated *Escherichia coli* F-18 and *E. coli* K-12. Infect. Immun. **57**:3022–3029.
35. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Minion, F. C., S. N. Abraham, E. H. Beachey, and J. D. Goguen.** 1986. The genetic determinant of adhesive function in type 1 fimbriae of *Escherichia coli* is distinct from the gene encoding the fimbrial subunit. J. Bacteriol. **165**:1033–1036.
37. **Neeser, J.-R., B. Koellreutter, and P. Wuersch.** 1986. Oligomannoside-type glycopeptides inhibiting adhesion of *Escherichia coli* strains mediated by type 1 pili: preparation of potent inhibitors from plant glycoproteins. Infect. Immun. **52**:428–436.
38. **Nishikawa, A., T. Sekine, R. Ikeda, T. Shinoda, and Y. Fukazawa.** 1990. Reassessment of antigenic determinants of *Saccharomyces cerevisiae* serotype Ia. Microbiol. Immunol. **34**:825–840.
39. **Ohman, D. E.** 1988. Experiments in gene manipulation. Prentice Hall, Inc., Englewood Cliffs, N.J.
40. **Ofek, I., and E. H. Beachey.** 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. **22**:247–254.
41. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Science **265**:623–625.
42. **Ofek, I., and N. Sharon.** 1990. Adhesins as lectins: specificity and role in infection. Curr. Top. Microbiol. Immunol. **151**:91–113.
43. **Old, D. C.** 1972. Inhibition of interaction between fimbrial haemagglutinins and erythrocytes by D-mannose and other carbohydrates. J. Gen. Microbiol. **71**:149–157.
44. **Orndorff, P. E., and S. Falkow.** 1984. Organization and expression of genes responsible for type 1 piliation in *Escherichia coli*. J. Bacteriol. **159**:736–744.
45. **Petersen, T. E., K. Skorstengaard, and K. Vibe-Pedersen.** 1990. Primary structure of fibronectin, p. 1–24. *In* D. Mosher (ed.), Fibronectin. Academic Press, New York.
46. **Quiocho, F. A.** 1986. Carbohydrate-binding proteins: tertiary structures and protein-sugar interactions. Annu. Rev. Biochem. **55**: 287–315.
47. **Rosenstein, I. J., M. Stoll, T. Mizuochi, R. Childs, E. Hounsell, and T. Feizi.** 1988. New type of adhesive specificity revealed by oligosaccharide probes in *E. coli* from patients with urinary tract infection. Lancet **ii**:1327–1330.
48. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
49. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
50. **Sharon, N., and I. Ofek.** 1986. Bacterial surface lectins specific for mannose, p. 55–81. *In* D. Mirelman (ed.), Microbial lectins and agglutinins. John Wiley & Sons, New York.
51. **Sokurenko, E. V., H. S. Courtney, S. N. Abraham, P. Klemm, and D. L. Hasty.** 1992. Functional heterogeneity of type 1 fimbriae of *Escherichia coli*. Infect. Immun. **60**:4709–4719.
51a. **Sokurenko, E. V., and D. L. Hasty.** Unpublished data.
52. **Strömberg, N., B.-I. Marklund, B. Lund, D. Ilver, A. Hamers, W. Gaastra, K.-A. Karlsson, and S. Normark.** 1990. Host-specificity of uropathogenic *Escherichia coli* depends on differences in binding specificity to Galα1-4Gal-containing isoreceptors. EMBO J. **9**:2001–2010.
53. **Strömberg, N., P.-G. Nyholm, I. Pascher, and S. Normark.** 1991. Saccharide orientation at the cell surface affects glycolipid receptor function. Proc. Natl. Acad. Sci. USA **88**:9340–9344.
54. **Takasaki, S., K. Yamashita, K. Suzuki, S. Iwanaga, and A. Kobata.** 1989. The sugar chains of cold-insoluble globulin, a protein related to fibronectin. J. Biol. Chem. **254**:8548–8553.
55. **Tewari, R., J. I. MacGregor, T. Ikeda, J. R. Little, S. C. Hultgren, and S. N. Abraham.** 1993. Neutrophil activation by nascent FimH subunits of type 1 fimbriae purified from the periplasm of *Escherichia coli*. J. Biol. Chem. **268**:3009–3015.
56. **Westerlund, B., P. Kuusela, T. Vartio, I. van Die, and T. K. Korhonen.** 1989. A novel lectin-independent interaction of P fimbriae of *Escherichia coli* with immobilized fibronectin. FEBS Lett. **243**:199–204.
57. **Westerlund, B., I. van Die, C. Kramer, P. Kuusela, H. Holthöfer, A.-M. Tarkkanen, R. Virkola, N. Riegman, H. Bergmans, W. Hoekstra, and T. K. Korhonen.** 1991. Multifunctional nature of P fimbriae of uropathogenic *Escherichia coli*: mutations in *fsoE* and *fsoF* influence fimbrial binding to renal tubuli and immobilized fibronectin. Mol. Microbiol. **5**:2965–2975.
58. **Zardi, L., B. Carnemolla, E. Balza, L. Borsi, P. Castellani, M. Rocco, and A. Siri.** 1985. Elution of fibronectin proteolytic fragments from a hydroxyapatite chromatography column. A simple procedure for the purification of fibronectin domains. Eur. J. Biochem. **146**:571–579.